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Applicants: Toru KIMURA et al.

Serial No.: 09/284,180 Group: 1633

Filed: June 9, 1999 Examiner: S. Chen

For: NOVEL SEMAPHORIN GENE: SEMAPHORIN W

## *DECLARATION UNDER 37 C.F.R. § 1.132*

Assistant Commissioner of Patents
Washington, D.C. 20231

March 28, 2001

Sir:

I, Toru KIMURA, residing at 1402, Noji-cho, Kusatsu-shi, Shiga, JAPAN, declare as follows:

1. I am a citizen of JAPAN.

2. I am currently employed in research activities at Sumitomo Pharmaceutical Company, Ltd. I am engaged in research on Semaphorin, and am responsible for the cloning of Semaphorin W and Semaphorin Y, in addition to Semaphorin Z. I am an author or co-author of 3 papers in this field.

3. I have read and understand the subject matter of U.S. Application Serial Number 09/284,180 and I am familiar with the prosecution of the application.

4. Together with Kaoru Kikuchi have carried out, or supervised, the experiments relating to the invention described below.

5. The following remarks are submitted to show that a DNA fragment as claimed in the above-identified application can be obtained without false results under the conditions and directions provided in the present specification.

6. The claims of the above-identified application are directed to isolated DNA that encodes a protein comprising a Semaphorin domain and/or that has at least one biological activity of a Semaphorin protein and that hybridizes under stringent conditions with the nucleotide sequence shown in one of SEQ ID NOS: 1, 4, 7 or 10. As recited on page 20 of the present specification, "stringent conditions" may include a formamide concentration of about 45% (v/v), a salt concentration of about 5X SSPE, and a temperature of about 42°C, and washing under the following conditions: a salt concentration of 2X SSPE, and a temperature of about 42°C.

7. The present specification provides guidance of how one skilled in the art would obtain a DNA as claimed. First, the 196 bp cDNA fragment as shown in SEQ ID NO:7 is used as a probe to screen a cDNA library. Screening of a cDNA library is well known in the art of molecular biology. Further, many different types of cDNA libraries exist and can be bought commercially. Therefore one of skill in the art would expect to find Semaphorin W protein-encoding nucleic acids in a cDNA library made from brain tissue.

8. Plaques obtained from the cDNA library can be transferred onto a nylon membrane, denatured, neutralized, and fixed to the membrane. Hybridization of the membrane and the 196 bp DNA fragment (SEQ ID NO:7) labeled with $^{32}P$ can be conducted at 42°C for 48 hours. The hybridization solution can be made up of hybridization buffer (45% (v/v) formamide, 5 x SSPE (1x SSPE consists of 0.15 M sodium chloride, 10 mM sodium dihydrogenphosphate, and 1 mM disodium ethylenediaminetetraacetate, adjusted to pH 7.0), 2x Denhardt's solution, 0.5% (w/v) sodium dodecyl sulfate (SDS), 20 μg/ml salmon sperm DNA.

9. After hybridization, the membrane can be washed 2-3 times in 2x SSPE, 0.5% (w/v) SDS at room temperature for 10 min, and further washed 2-3 times in 2x SSPE, 0.5% (w/v) SDS at 42°C for 10 min. The membranes are exposed to film and any positive signals may be further researched. Plaques corresponding to positive signals are excised from the agarose plate from which they originated, placed in 500 μl of SM buffer (100 mM sodium chloride, 15 mM magnesium sulfate, 50 mM Tris (pH 7.5), 0.01% gelatin) supplemented with 20 μl of chloroform, and left overnight at 4°C to elute the phages.

10. The recombinant lambda phages thus obtained can be subjected to a secondary screening according to the same procedures in order to isolate single plaques.

11. The phages thus obtained can be treated in the following manner for *in vivo* excision of a phagemid containing the cDNA insert, according to the protocols supplied by Stratagene.

12. Agarose gels containing the plaques obtained in the secondary screening are placed in 500 μl of SM buffer, supplemented with 20 μl of chloroform, and then allowed to stand overnight at 4°C. Two hundred fifty μl of the phage solution obtained, 200 μl of *E. coli* XL-1 Blue MRF' suspended in 10 mM magnesium chloride at $OD_{600}$=1.0, and 1 μl of ExAssist helper phage (>1x$10^6$ pfu/ml) are mixed, and incubated at 37°C for 15 min. Then, 3 ml of LB medium (prepared by mixing 0.5% (w/v) sodium chloride, 1% (w/v) Bactotrypton, and 0.5% (w/v) yeast extract and then adjusting the mixture to pH 7.0 using 5 M sodium hydroxide) is added, and the mixture is shaken at 37°C for 2-3 hours. The cells can be removed by centrifuging at 2000xg for 15 min, and the supernatant is treated at 70°C for 15 min. The supernatant is then centrifuged again at 2000xg for 15 min, and the supernatant is recovered as a stock solution of a phagemid containing the cDNA insert.

13. An aliquot (10-100 μl) of the phagemid stock solution is mixed with 200 μl of *E. coli* SOLR ($OD_{600}$=1.0), incubated at 37°C for 15 min, and 10-50 μl of the mixture is then plated onto an ampicillin plate, incubated overnight at 37°C to obtain *E. coli* strain containing a double stranded phagemid into which the gene fragment of interest has been inserted.

14. The nucleotide sequence of the cDNA clone thusly obtained can be then analyzed on, for example, a Model 377 DNA Sequencer (Perkin-Elmer) to determine the total nucleotide sequence.

15. Next, the sequence of the gene thusly obtained can be translated into an amino acid sequence and compared to the Semaphorin domain amino acid sequence. If the novel protein has the Semaphorin amino acid domains, then the novel protein belongs to the Semaphorin protein family. Finally, once the protein is determined to contain a Semaphorin domain, the elements of claim 36 are met, i.e. an isolated DNA that encodes a protein comprising a Semaphorin domain and which hybridizes under stringent conditions with the nucleotide sequence shown in SEQ ID NO:7.

16. The other nucleic acids recited in the claims (SEQ ID NOS:1, 4 and 10) can be used in a similar manner as described above for the nucleic acid of SEQ ID NO:7.

17. Furthermore, once the practitioner has in hand a nucleic acid encoding a Semaphorin domain-containing protein, that nucleic acid can be expressed and tested for biological activity using the assay described in Examples 8 and/or 9 of the specification, or alternatively one of the bioassays known in the art at the time the present application was filed (e.g. as described in Exhibit 3 attached to the Amendment responsive to the Office Action of September 28, 2000).

18. The experimental approaches described above were used to isolate cDNA encoding rat Semaphorin W (SEQ ID NO: 1) using the 196 nucleotide DNA of SEQ ID NO: 7. The human cDNAs encoding C- and N-terminal portions of human Semaphorin W (SEQ ID NOS:4 and 10, respectively) were isolated using the above experimental approaches and using the rat Semaphorin cDNA of SEQ ID NO:2 as a probe. These data establish that the procedures described in the specification for isolating Semaphorin W-encoding nucleic acids can be utilized to obtain additional embodiments of the invention without experimentation considered undue in the art of molecular neurobiology.

I hereby declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated this _____ day of March, 2001.

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Toru Kimura, Ph.D.